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Exogenous Melatonin Improves the Quality Performance of Rice under High Temperature during Grain Filling

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Citation: Fan, X.; Zhao, J.; Sun, X.; Zhu, Y.; Li, Q.; Zhang, L.; Zhao, D.; Huang, L.; Zhang, C.; Liu, Q. Exogenous Melatonin Improves the Quality Performance of Rice under High Temperature during Grain Filling. *Agronomy* **2022**, *12*, 949. https://doi.org/10.3390/agronomy12040949

Academic Editor: Alessio Aprile

Received: 12 March 2022
Accepted: 12 April 2022
Published: 15 April 2022

Abstract: With the increasing greenhouse effect, high temperature has become the most unfavorable environmental factor for the rice grain filling process, affecting rice yield and quality mainly through changing the composition and structure of starch in rice grains. Research has focused on the rational management of water and fertilizer, and spraying of exogenous chemicals, which have become important measures to alleviate high temperature stress of rice. As a multifunctional molecule, melatonin has the potential to improve plant stress resistance by enhancing the scavenging efficiency of reactive oxygen species (ROS), thus protecting plants from the adverse effects of abiotic stress. The present study used a typical *japonica* rice variety Nipponbare (NPB) as the experimental material, which was treated with high temperature and melatonin during grain-filling stages. The effects of exogenous melatonin on the rice growth and quality traits, as well as starch synthesis, in response to high temperature were analyzed systematically. Exogenous melatonin significantly increased the rice leaf photosynthetic and heat-resistance properties. Melatonin could alleviate the effects of high temperature on the key physicochemical properties related to rice quality. Furthermore, milled rice from NPB plants treated with melatonin had better endosperm appearance under high temperature. Further study found that exogenous melatonin could stabilize the chain length distribution of starch in NPB (especially amylopectin), which implied that melatonin could be used in rice cultivation to alleviate the effect of high temperature on quality, optimization of amylopectin synthesis can also improve rice quality. The results of the present study provide a new idea and research direction to alleviate high temperature stress of rice in the context of global warming.

Keywords: high temperature; rice (*Oryza sativa* L.); melatonin; grain quality

1. Introduction

Rice is one of the most widely planted staple crops in the world. Rice production plays an important role in ensuring food security. With the gradual improvement of living conditions, people's demand for rice is gradually increasing. Therefore, it is necessary for breeders to cultivate high-quality rice varieties with good appearance, better taste and rich nutrition [1]. With the increasing greenhouse effect, high temperature weather has occurred frequently in recent years. High temperature induces plant cells to produce reactive oxygen species (ROS), including H_2O_2, O_2^-, and OH^-, which further leads to oxidative stress and damage to cellular membranes, constituents, and macromolecules, as well as disrupting metabolic functions [2]. The efficiency of the antioxidant system in plants directly determines the ability of plants to scavenge excessively accumulated ROS under high temperature stress and restores the damage induced by ROS, which also affects plants' resistance to high temperature [3]. Studies have shown that a temperature above 35 °C not only affects the flowering and pollination of rice, leading to a decline in yield, but also leads to the degradation of rice quality in the grain-filling period. High temperature can reduce the accumulation of starch and other organic matter, eventually leading to decreased yield and poor-quality characteristics of rice, such as insufficient grain filling and increased chalkiness. During subsequent processing, the broken rice rate is greatly increased, resulting in poor appearance and eating quality [4].

The rice seed endosperm is the main edible part of rice (more than 90% of the weight of brown rice), and starch is the most important component of the rice endosperm (more than 90% of the dry weight of endosperm). Therefore, the starch synthesized during the development of the rice endosperm is an important component of human food. The composition and structure of starch are the most important factors that determine the quality and applicability of rice [5]. Starch is mainly synthesized in endosperm cells, and the process of starch accumulation is synchronous with their development. Rice starch is mainly composed of amylose and amylopectin. Amylose is a linear glucose polymer linked by α-1,4 glycosidic bonds that has few branches, while amylopectin is synthesized based on amylose and linked branched chains by α-1,6 glycosidic bonds [6]. The molecular weight of amylopectin is much higher than that of amylose. The chain length distribution of different lengths of starch in rice directly affects the processing characteristics, eating quality, and applicability of rice. The amylose content (AC) is one of the important indicators to judge the quality of rice, which is closely related to the cooking and eating quality of rice [7]. Cooked rice containing a higher AC has lower viscosity, larger swelling capacity, lower glossiness, and increased hardness after cooling, resulting in poor appearance and taste quality. However, cooked rice with a lower AC has higher viscosity, less swelling, and more luster after cooking, showing a better appearance and eating quality [1]; therefore, consumers prefer rice containing a medium low AC.

Environmental factors such as temperature, light, soil conditions and precipitation can affect the quality of rice to different degrees, among which temperature has the most obvious effect on rice quality [8]. The heading and filling process of rice is the key period in the formation of rice quality, and a high temperature during the grain-filling period has a significant effect on rice quality. At present, the effects of high temperature on grain filling and chalkiness of rice have been clarified [9]. Under high temperature stress, the photosynthetic efficiency of rice decreases and the grain filling rate accelerates. The most important physiological cause leading to a decrease in rice quality is the effect of high temperature on the metabolism of endosperm storage substances, especially starch [10]. There are many reports on heat stress leading to chalky grain production due to the changes in starch synthesis [11]. High temperature usually decreased amylose content (AC) and adjusted gelatinization temperature (GT) in many rice varieties [12]. Reduction of AC directly affects the variation between translucent and chalky grains [13], which is the main reason of the alterations in starch structure under high temperature. In the chalk particles, there are many hollows in the loosely stacked powder particles, which hinder the transmission of light, and opaque areas can be seen along the translucent particles [14]. In addition, high temperature not only reduces AC but it also increases the overall proportion of amylopectin with longer chains in rice starch [11]. It is concluded that high temperature can change the quality of rice, especially the appearance quality, by affecting starch structure and chain length distribution.

In the context of global warming, rice breeding and production is presented with new challenges and demands. In the face of uncontrollable environmental conditions, how can we enhance the ability of rice to resist stress? How can we maintain the good quality of rice while ensuring the yield of rice? The current control measures used to reduce high temperature stress of rice mainly include reasonable fertilizer and water management, adjusting the sowing date, and cultivating heat-resistant varieties. In addition, previous studies have shown that some exogenous chemicals can improve the growth status and stress resistance of plants, one of which is melatonin. Melatonin (N-acetyl-5-methoxytryptamine) is an indole hormone produced by the pineal gland of vertebrates, which was first isolated from the bovine pineal gland [15]. Studies have shown that it has many biological functions in animals, including regulation of circadian rhythm, sleep, body temperature, appetite, retinal physiology, immune system and sexual behavior [16,17]. Subsequent studies have found that melatonin is also common in higher plants. It has been confirmed that melatonin exists in more than 140 diverse plant species [18]. Melatonin is preferentially synthesized in the reproductive organs of plants, and the content of melatonin in flowers and seeds is much higher than that in other tissues [19]. Plant melatonin participates in a variety of physiological functions, such as regulating the growth of roots, buds and explants [20]; photosynthesis [21]; activating seed germination and rooting; delaying leaf senescence [22]; and alleviating the damage caused by biotic and abiotic stresses [21]. Furthermore, exogenous melatonin has been shown to confer plant tolerance to a variety of environmental stresses, such as heavy metal (Cd, Cu), salt, cold, drought, and pathogen infection [23,24]. Further studies indicated that melatonin could act as an antioxidant, and has important functions, such as scavenging reactive oxygen species and reactive nitrogen species (ROS and RNS), chemical detoxification, and repairing membrane stability and membrane fluidity [25]. Recent RNA-sequencing results indicated that melatonin could improve the resistance of cotton to salt stress by adjusting antioxidant enzymes, transcription factors, plant hormones, signal molecules, and Ca^{2+} signal transduction [26]. Although the pathway of exogenous melatonin into plants is not clear, studies have shown that exogenous melatonin can participate in abiotic stress response of plants by regulating many endogenous hormones including melatonin [27,28].

In this study, typical japonica rice (NPB) was cultivated in climate chamber and treated by high temperature at the grain filling stage, and exogenous melatonin treatment was provided at the same stage. The quality characteristics of rice were investigated after the rice matured, and the improving effect of melatonin on rice starch quality-related properties in response to high temperature was analyzed systematically. This study could provide new ideas and research directions for the production with good quality rice under high temperature conditions.

2. Materials and Methods

2.1. Plant Materials and Stress Treatments

The *japonica* rice variety Nipponbare (NPB) was used as treatment material in this study. Each 2 plants were cultivated in 20 L bucket under natural environment conditions until the booting stage, then all the plants were transferred into controlled environment growth chambers to adapt to the controlled environment in advance. The used soil is taken from the rice experimental field of Yangzhou university, which is divided into buckets after manual mixing with urea (1 g urea/3 kg soil). Before flowering, all rice materials were cultivated in the artificial climate chamber at normal temperatures (32 °C/22 °C, 2.38 kPa/1.32 kPa, day/night), while other environmental conditions were set as follows: humidity 50% and light: dark = 14 h:10 h (Supplementary Materials Figure S1). At 5 days after flowering (DAF), half of these materials were transferred into the artificial climate chamber under high temperature (38 °C/28 °C, 3.31 kPa/1.89 kPa, day/night). The other environmental conditions in the artificial climate chamber were the same as those for the plants cultivated at normal temperature. During temperature treatment, each plant was also treated by 250 mL of 200 μmol L^{-1} melatonin (Sigma-Aldrich, St. Louis, MO, USA) at 10 DAF, 15 DAF and 20 DAF. The control group was sprayed with the same volume of dH_2O.

2.2. Flower Tagging and Sampling

The exact flowering time of rice was determined by using the flower marking method. Flag leaves at 5 days after treatment (5 DAT) were sampled to determine the relative indexes of photosynthetic efficiency and stress resistance. Mature seeds harvested from rice materials planted under different temperatures and treated by different exogenous substances were collected to analyze yield and starch-related quality.

2.3. Analysis of Photosynthetic and Stress Resistance Characteristics of Leaves

A high-speed continuous excitation fluorometer (Pocket PEA, HANSATECH, King's Lynn, UK) was used to measure the character of rapid chlorophyll fluorescence in rice flag leaves at 5 DAT. Fv/Fm represents primary conversion efficiency of photosystem II, and the performance index (PI) represents the photochemical index, which is another characterization of the overall function of photosystem II. The activities of catalase (CAT) and total superoxide dismutase (T-SOD), as well as contents of hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) were determined using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Preparation of Seed Samples and Starch Isolation

After being air-dried, rice seeds were dehusked using a rice huller (SY88-TH, Sangyong Ltd., Incheon, Korea), polished using a rice grain polisher (Kett, Tokyo, Japan). The chalkiness degree of rice grain with different treatments was identified by an appearance detection analyzer (MRS-9600TFU2L, MICROTEK, Shenzhen, China). Then rice grains were ground in a mill (FOSS 1093 Cyclotec Sample mill, FOSS, Hilleroed, Denmark) with a 0.5 mm screen. Ten grams of the polished rice seeds were soaked in three volumes of Tris-HCl solution (50 mM Tris-HCl, 10 mM $CaCl_2$, pH 7.0). After standing overnight at room temperature, these polished seeds were grated using a waring blender (IKA-RCT-Basic, KA®-Werke GmbH & Co. KG, Staufen, Germany) at 1500 rpm for 3 min. Rice milk was added with 5 mg of protease K (Amresco, Radnor, PA, USA) and mixed on a magnetic stirrer at 37 °C for 24 h. The mixture was filtered through a 75 µm filter and centrifuged at $3600\times g$ for 20 min. After removing the supernatant, the sediment was washed with dH_2O and centrifuged for 15 min, which was repeated for 5 times. Washed starch samples were dried in a convection oven at 40 °C for 48 h.

2.5. Total Starch Content and Starch Fine Structure Analysis

According to the manufacturer's protocol, the total starch content in rice flour was tested using a Total Starch Assay Kit (Megazyme, Bray, Ireland). The starch fine structure was analyzed by using size-exclusion chromatography (SEC) and high-performance anion exchange chromatography (HPAEC) methods. A 6–8 mg starch sample was dissolved in 1.8 mL of dimethyl sulfoxide (DMSO: $NaNO_3$ = 1:1), which was shaken at 600 rpm at 80 °C overnight and centrifugated at 4000 rpm for 10 min. Then, 1.5 mL of the supernatant was mixed with 6 mL of absolute ethanol for 15 min and then centrifuged at the same speed for 10 min. After the supernatant was removed, the sediment was washed with 6 mL of 85% ethanol and dried for approximately 30 min. The purified starch was de-branched and lyophilized according to a previously described method. An Agilent 1100 Series SEC system (Agilent Technologies, Waldbronn, Germany) equipped with GRAM 100 and GRAM 1000 analytical columns (Polymer Standards Service (PSS), Mainz, Germany) set at 80 °C and a differential refractive index (DRI) detector (Wyatt, Santa Barbara, CA, USA) was used to carry out the analysis of chain length distribution of amylose, which can measure the AC accurately. A HPAEC instrument (ICS-5000A, Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the branch chain length distributions (CLDs) of amylopectin.

2.6. RVA and DSC Analysis

The pasting properties of rice flour were investigated using a Rapid Visco-Analyser (RVA) (Newport Scientific, Warriewood, Australia) according to previously described protocols [5]. Differential scanning calorimetry (DSC, DSC 200F3, Netzsch Instruments NA LLC, Burlington, MA, USA) was used to identify the gelatinization temperature based previously published methods. The DSC curves were used to calculate the following parameters: the temperature of onset (T_0), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH).

2.7. Statistical Analysis

At least three biological replicate samples (from NPB plant grown in different bucket) were used for three technical determinations to characterize the samples. Data represent the mean values plus the standard deviation (SD) of at least three independent experiments. Values with different letters in the same column are significantly different at $p < 0.05$, calculated by one-way ANOVA.

3. Results

3.1. Exogenous Melatonin Enhanced the Resistance of Rice to High Temperature Stress

The flag leaf (10 DAF/5DAT) from rice plants treated with exogenous melatonin was brighter green than that of rice plants treated with dH_2O (Figure 1A), which indicated that melatonin could maintain the stability of chloroplasts and delay the senescence process of rice leaves. Furthermore, the photosynthetic indexes (Fv/Fm, PI) of the flag leaf treated with melatonin were significantly higher than those of the control under both temperatures (Figure 1B,C). Thus, exogenous melatonin treatment could better maintain the stability of the photosynthetic system in rice leaves, and the photochemical activity of photosystem II in leaves treated with melatonin was higher. To further explore the effect of melatonin on rice growth in response to high temperature, we measured the activities of enzymes related to antioxidant stress in plants (CAT and T-SOD). The results indicated that high temperature upregulated the activity of CAT and downregulated T-SOD activity of NPB, exogenous melatonin treatment further upregulated the activities of these antioxidant enzymes (Figure 1D,E). It should be noted that exogenous melatonin treatment also increased the activities of two antioxidant enzymes at normal temperature, and the upregulation of CAT activity was more significant, which suggested that exogenous melatonin treatment caused a certain degree of oxidative stress response under normal temperature.

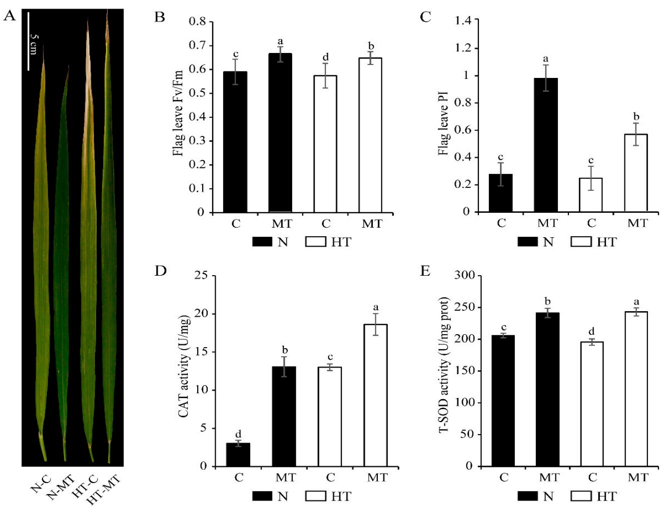

Figure 1. Photosynthetic indexes of NPB plants treated with melatonin at different temperatures. (**A**) Photographic observation of NPB rice leaves. (**B**,**C**) Determination of Fv/Fm and PI values of flag leaves of NPB. (**D**,**E**) Determination of CAT and T-SOD activities of NPB leaves. The data presented in the figure are repeated through three independent experiments, values with different letters (a, b, c and d) in the same column are significantly different at $p < 0.05$, calculated by one-way ANOVA. "N" and "HT" indicate normal and high temperature conditions. "C" and "MT" indicate H_2O and melatonin treatment, respectively.

3.2. Exogenous Melatonin Improves the Grain Appearance Quality

One of the most direct effects of high temperature on rice quality is the significant increase of seed chalkiness (Figure 2A). Although melatonin did not influence the degree of chalkiness of NPB grains under normal temperature, melatonin significantly reduced the proportion of chalky rice from 14.46 to 7.77%. High temperature caused the degree of chalkiness of NPB grains to increase from 2.28% to 55.28%, and increased the proportion of chalky rice from 14.46 to 85.40%. Melatonin did not influence the proportion of chalky rice of NPB grains under high temperature, but reduced degree of chalkiness of NPB grain from 55.28 to 43.22% (Figure 2B,C). Thus, it can be seen that melatonin could significantly improve the appearance of rice seeds under high temperature.

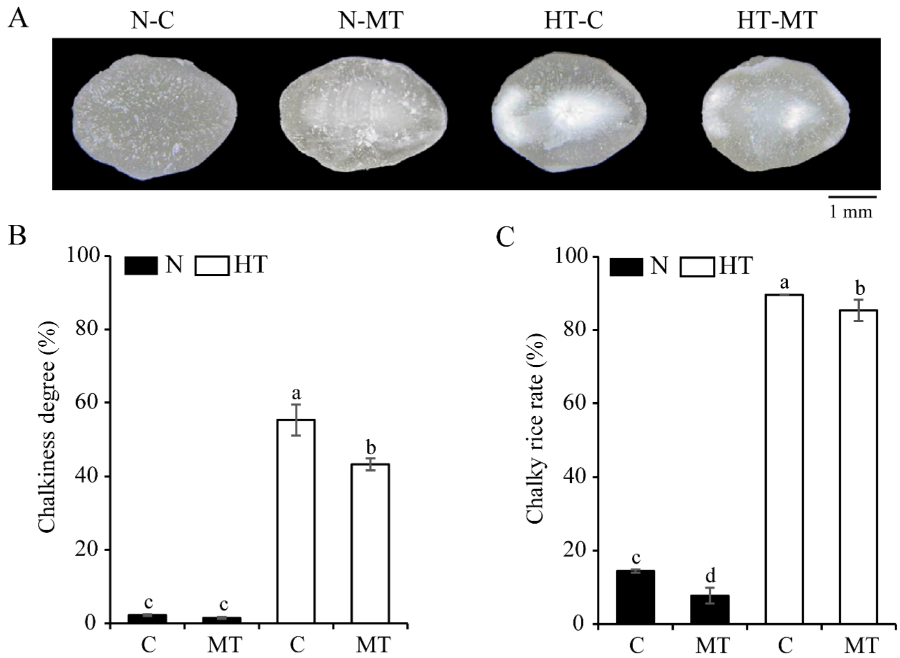

Figure 2. Effects of melatonin treatment on the appearance quality of NPB grain at different temperatures. (**A**) Photographic observation of cross sections of NPB grains with different treatments. (**B**) Chalkiness degrees of NPB grains with different treatments. (**C**) Chalky grain rate of NPB grains with different treatments. The data presented in the figure are repeated through three independent experiments, values with different letters (a, b, c and d) in the same column are significantly different at $p < 0.05$, calculated by one-way ANOVA. "N" and "HT" indicate normal and high temperature conditions. "C" and "MT" indicate H_2O and melatonin treatment, respectively.

3.3. Effect of Exogenous Melatonin on Starch Synthesis in Endosperm

Scanning electron microscope (SEM) observation of seed cross section and starch grains indicated that the increase in chalkiness of seeds induced by high temperature treatment affects the synthesis and accumulation of starch in the seeds of NPB. In the absence of melatonin treatment, high temperature led to the reduction of starch particle size and irregular structure in NPB seeds. Moreover, high temperature treatment resulted in many pores on the surface of starch grains. From the cross-sectional scanning results, it can be seen that the grain filling is not full due to high temperature, the starch grains cannot form regular crystals, and many pores are produced at the same time. Although melatonin treatment did not protect the starch particle size from the influence of high temperature, it could maintain the regularity of starch particle structure, which ensured that the starch grains form regular crystals and were not easy to form pores in or between the starch grains (Figure 3 and Supplementary Materials Figure S2). High temperature significantly decreased the total starch content and amylose content in NPB grains, melatonin treatment alleviated the inhibition of high temperature on amylose synthesis to a certain extent

(Figure 4A,B). HPAEC method was used to identify the fine structure of the starch of grains from NPB under different treatments. Result of HPAEC indicated that high temperature markedly increased the content of amylopectin with short chains (DP 6–12), but decreased the content of amylopectin with long chains (DP 13–60). Melatonin significantly reduced the effect of high temperature on amylopectin synthesis with short chains (DP 6–22), and it stabilized the synthesis of amylopectin with longer chains (DP 22–60) which were hardly affected by high temperature (Figure 4C).

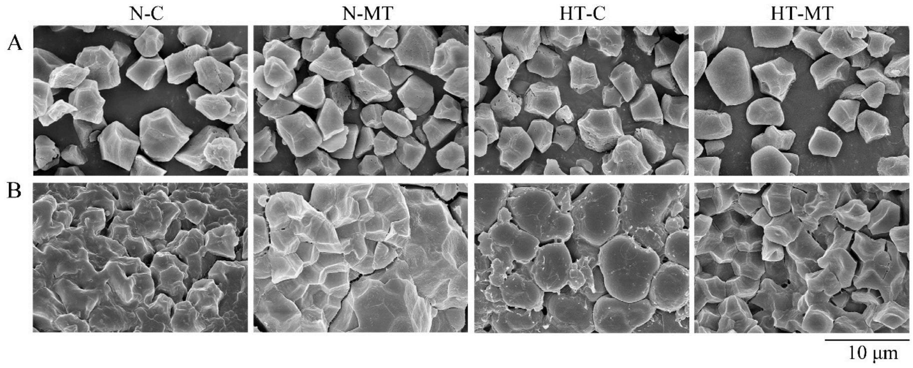

Figure 3. SEM observation of starch granule (**A**) and grain cross-section (**B**) of NPB grown at different temperatures. "N" and "HT" indicate normal and high temperature conditions. "C" and "MT" indicate H_2O and melatonin treatment, respectively.

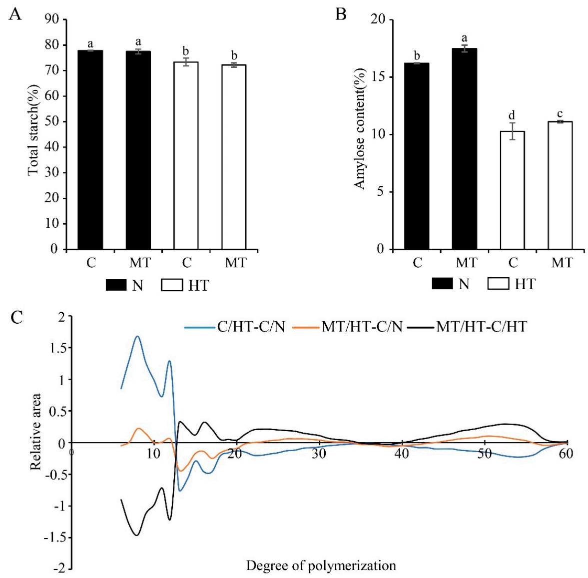

Figure 4. Effects of melatonin treatment on the starch synthesis of NPB grown at different temperatures. (**A**) Total starch content of NPB grains with different treatments. (**B**) Amylose content of NPB grains with different treatments. (**C**) HPAEC analysis of NPB grains with different treatments. The data presented in the figure are repeated through three independent experiments, values with different letters (a, b, c and d) in the same column are significantly different at $p < 0.05$, calculated by one-way ANOVA. "N" and "HT" indicate normal and high temperature conditions. "C" and "MT" indicate H_2O and melatonin treatment, respectively.

3.4. Effect of Exogenous Melatonin on Pasting Properties of Rice in Response to High Temperature

Pasting properties is a comprehensive index to evaluate rice eating and cooking quality (ECQ), which is affected by the starch fine structure (chain length distribution of amylopectin and amylose size). RVA parameters include peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), peak time (PeT) and pasting temperature (PaT), which can be further used to calculate the breakdown viscosity (BDV) and setback viscosity

(SBV). RVA parameters were listed in Supplementary Materials Table S1. Under normal temperature, melatonin treatment significantly reduced the values of viscosity-related parameters (PKV, HPV, CPV, BDV, and SBV). Peak time (PeT) of NPB was not affected by melatonin, but melatonin increased the pasting temperature (PaT) of NPB remarkably (Figure 5A). Without the melatonin treatment, high temperature significantly increased the values of PKV, HPV, CPV, and SBV, but decreased the value of BDV. In addition, high temperature extended peak time of NPB and raised the pasting temperature dramatically. However, melatonin can restore pasting properties of NPB to normal temperature to a large extent (Figure 5B).

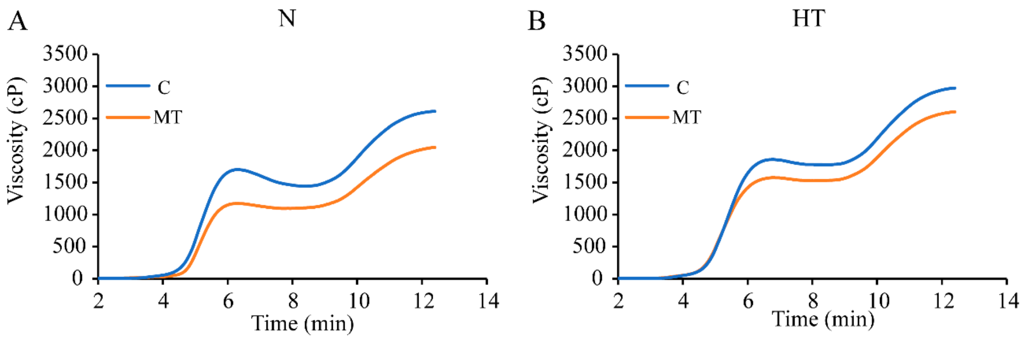

Figure 5. Effects of melatonin treatment on the pasting properties of NPB grown at different temperatures. (**A**,**B**) RVA analysis of NPB treated with exogenous substances under normal and high temperature. The data presented in the figure are repeated through three independent experiments. “N” and “HT” indicate normal and high temperature conditions. “C” and “MT” indicate H_2O and melatonin treatment, respectively.

3.5. Effect of Exogenous Melatonin on Gelatinization Temperature of Rice in Response to High Temperature

Gelatinization temperature (GT) is the temperature at which birefringence and crystallinity disappear during rice gelatinization, which is usually determined by DSC. A series of parameters including onset temperature (T_0), peak temperature (T_m) and conclusion temperature (T_c), as well as gelatinization enthalpy (ΔH) are used to quantify gelatinization. Under normal temperature, melatonin treatment didn’t influence the gelatinization characteristics of NPB at all. Without the melatonin treatment, high temperature significantly raised onset temperature, peak temperature, and conclusion temperature of NPB, but it didn’t affect the gelatinization enthalpy of NPB. Interestingly, melatonin treatment did not change the effect of high temperature on gelatinization characteristics of NPB (Figure 6 and Supplementary Materials Table S2).

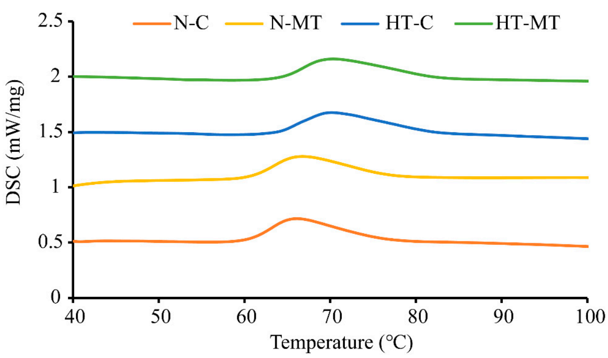

Figure 6. DSC analysis of NPB treated with exogenous substances under normal and high temperature. The data presented in the figure are repeated through three independent experiments. “N” and “HT” indicate normal and high temperature conditions. “C” and “MT” indicate H_2O and melatonin treatment, respectively.

4. Discussion

4.1. Melatonin Enhanced the Antioxidant Capacity of Rice Leaves under High Temperature Stress

As the source tissue, rice leaves can absorb carbon dioxide (CO_2) for photosynthesis and provide energy for plant growth and development. The antioxidant capacity and photosynthetic efficiency of leaves directly affect all the physiological and biochemical process of rice plants. The influence of high temperature on rice yield and quality results from the excessive production of ROS in rice cells. ROS are the by-product of photosynthesis, respiration and other normal metabolic processes, and play an important role in stress tolerance [29]. Although ROS are involved in programmed cell death, abiotic stress responses and virus defense [30], the excessive accumulation of ROS can stimulate membrane lipid peroxidation, leading to cell membrane damage, loss of cell integrity and cell death [31]. Plants have evolved enzyme or non-enzyme antioxidant systems to maintain the redox balance among enzyme antioxidant defense systems. Superoxide dismutase (SOD) is an antioxidant enzyme that catalyzes the disproportionation of superoxide anion radicals (O_2^-) to produce oxygen (O_2) and hydrogen peroxide (H_2O_2), playing a crucial role in the balance of oxidation and antioxidation. Catalase (CAT) is an enzyme scavenger, which binds to iron porphyrin as an auxiliary group. CAT promotes the decomposition of H_2O_2 into molecular oxygen (O_2) and water (H_2O), removing the hydrogen peroxide in the body, thus protecting cells from H_2O_2 toxicity. CAT is one of the key enzymes in biological defense systems. The mechanism of CAT's effect on H_2O_2 is essentially the disproportionation of H_2O_2. Consistent with the results of previous studies, our research indicated that exogenous melatonin treatment could improve the antioxidant capacity of rice leaves. Specifically, melatonin increased the activities of T-SOD and CAT in flag leaves from NPB plants. In addition, melatonin increased the activities of these two enzymes to a greater extent under high temperature (Figure 1D,E). Stronger antioxidant capacity in the rice leaves treated with melatonin resulted in a better photosynthetic capacity, thus is that the leaves treated with melatonin had more fresh green appearance, and the values of photosynthesis related parameters (Fv/Fm, PI) were higher (Figure 1B,C).

4.2. Melatonin Stabilized the Starch Synthesis in Rice under High Temperature

Previous studies showed that melatonin enhanced instant starch accumulation by regulating the related gene expression and the activity of α-amylase and starch phosphorylase, which also helped to improve arsenic stress tolerance. This demonstrated the efficacy of melatonin in regulating the metabolism of starch and sugar, which would enhance tolerance to abiotic stress. In the present study, we focused on starch accumulation in the rice grains, which is directly related to yield and quality. The application of melatonin enhanced the antioxidant and photosynthetic capacity of leaves, but did not slow down the decrease in the total starch content under high temperature (Figure 4A). The decrease in the total starch content also led to a decrease in rice grain weight (Supplementary Materials Figure S3). Although melatonin did not influence the degree of chalkiness of NPB grains under normal temperatures, it significantly reduced the proportion of chalky rice of NPB grains. High temperature increased the degree of chalkiness and the chalky rice proportion of NPB grains. Melatonin did not influence the proportion of chalky rice of NPB grains under high temperature, but markedly reduced degree of chalkiness of NPB grains by about 10% (Figure 2B). Thus, melatonin improved the appearance of grains from NPB rice. Further study indicated that melatonin stabilized the starch synthesis in NPB rice under high temperature, although melatonin treatment alleviated the inhibition of high temperature on amylose synthesis to a certain extent (Figure 4B), melatonin protects against the effects of high temperature on amylopectin synthesis to a greater extent (Figure 4C). Therefore, adjustment in the fine structure of amylopectin can also affect the transparency of rice seeds, although more studies have shown that changes in amylose content have a significant effect on the transparency of rice seed. Our previous research results indicated that amylose and amylopectin are in a dynamic balance in the synthesis process at room temperature. High temperature will inhibit the synthesis of amylose, and then breaks the balance of amylose and amylopectin synthesis, and abnormal starch synthesis resulted in the decline of rice quality [6]. Melatonin treatment stabilized the synthesis of amylopectin to the greatest extent, which was easiest to affected by high temperature treatment. It is concluded that the adjustment of amylopectin synthesis may also be applied to rice quality improvement. In addition, several other physicochemical properties, including starch viscosity, gelatinization properties, and crystallinity were investigated. Melatonin can stabilize the viscosity and crystallinity of starch, but has little effect on the gelatinization temperature.

4.3. The Future Application of Melatonin in Rice Cultivation

Considering that the temperature treatment used in this study was extremely high during the whole filling stage, the concentration of melatonin used was relatively high compared with that used in previous studies. We found that high temperature irreversibly reduced the accumulation of related storage substances in seeds, and the content of total starch decreased significantly, which led to decreased grain weight. Melatonin treatment significantly improved the antioxidant and photosynthetic capacity of rice plants, which greatly reduced the effect of high temperature on the physical and chemical characteristics related to rice quality. Furthermore, melatonin improved the appearance quality of NPB grains under both normal and high temperature, which was surprising. This suggested that melatonin has great application prospects for quality improvement of non-glutinous rice under both normal and high temperature. As the greenhouse effect becomes increasingly severe, the frequency of extreme high temperature weather becomes unpredictable and more frequent. Since the high-temperature treatment in this study runs through the whole period of seed filling, the application amount of melatonin (three times of 500 μm melatonin spraying on a single rice plant) is slightly higher than that used in the previous study [32]. However, since melatonin is a highly degradable plant hormone, such application amount should not cause obvious environmental risk. It is worth noting that the application scheme of melatonin in this study improved the appearance of seeds, but also reduced the yield of seeds to a certain extent. We speculate that the treatment of exogenous melatonin makes the plant mobilize more energy to improve the activity of antioxidant enzyme system, which affected the accumulation of substances (such as starch) in seeds. Therefore, the application amount of melatonin in practical application can be adjusted according to the growth environment of crops and the intensity and duration of stress. In addition, this study showed that melatonin can stabilize the amylopectin synthesis of typical *japonica* rice. Therefore, melatonin can be used widely in the cultivation of various rice varieties to alleviate the effect of high temperature on quality. In addition, many studies have shown that plants (e.g., rice) can improve the state of their cells under stress by increasing the synthesis of endogenous melatonin, and exogenous melatonin can stimulate the synthesis of endogenous melatonin [33]. These observations indicate that the cultivation of rice varieties with an appropriately high melatonin content is another important measure that could be used to deal with various adverse natural environment in the future.

5. Conclusions

Our research was the first to investigate the effects of melatonin on the formation of starch synthesis related quality traits of rice under high temperature, and to clarify the effects of melatonin on starch synthesis related quality traits of rice in response to high temperature. The results provide new ideas and research directions for reducing high temperature stress of rice under global warming environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12040949/s1, Figure S1: Artificial climate chamber condition setting for temperature treatment; Figure S2: Distribution analysis of starch particle size of NPB under different treatment conditions; Figure S3: 1000-grain weight of NPB planted with different treatment conditions; Table S1: RVA parameters of NPB treated with exogenous substances at different temperatures; Table S2: DSC parameters of NPB treated with exogenous substances at different temperatures.

Author Contributions: Investigation, X.F., J.Z., X.S. and Y.Z.; data curation, X.F., J.Z., X.S. and Y.Z.; writing—review & editing, Q.L. (Qianfeng Li), L.Z., D.Z., L.H., C.Z. and Q.L. (Qiaoquan Liu); supervision and project ad-ministration, Q.L. (Qiaoquan Liu); funding acquisition, Q.L. (Qiaoquan Liu) and X.F.; Formal Analysis, Y.Z., X.S., J.Z. and X.F.; writing—original draft, X.F. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Science Foundation of China: 32072032; Projects from the government of Jiangsu Province: BZ2021017, CX(21)3010, BE2020318-1, BK20200045, and KYCX21_3246.

Data Availability Statement: Not applicable.

Acknowledgments: We sincerely thank Jindong Wang for their help in data analysis.

Conflicts of Interest: The authors declare no conflict of interest.

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